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Assessment of bioactive constituents present in sea buckthorn byproducts and their *in vitro* antioxidant potential

Arti Ghabru, C Varshneya, Neerja Rana, Geeta Verma and Shivani Chauhan

Abstract
Seabuckthorn (*Hippophae rhamnoides L*) has recently gained interest for its nutritional and medicinal values. Fruits and leaves are considered to be good source of large number of bioactive substances such as vitamins, trace elements, amino acids, β-carotene, zeaxanthin, lycopene, flavonoids, folic acid, fatty acid, tannic acid etc. Chemoprofiling (Total phenols, total flavonoids, vitamin C, vitamin E, lycopene and β-carotene contents) of various seabuckthorn byproducts has been done and it was found that among all the seabuckthorn byproducts, leaf extract contained significantly highest amounts of total phenols (332.49±7.45 mg/g), total flavonoids (271.56±5.41 mg/g), vitamin C (399.49±4.90 mg/100g) and lycopene content (8.50±2.92 mg/100g).
The proximate analysis of nutritive contents of Seabuckthorn byproduct was determined. The ash content which is an index of mineral contents, ranged from 1.3 to 4%. The moisture content was significantly highest in seedcake. The crude protein contents ranged from 13.89 to 23% and recorded highest in leaves (22.09%) and seedcake (23%). Ether and crude fibre contents were recorded highest in pomace with seeds. High concentrations of sodium (Na) were present, ranging from 40 to 160 mg/g. Among all the byproducts; leaves contained high concentration of all the minerals estimated.
Seabuckthorn byproducts were screened for the presence of antioxidant potential for inhibiting the different *in vitro* free radicals. The inhibition of the free radicals *i.e.* ABTS, DPPH, superoxide, hydroxyl and nitric oxide radicals by all the byproducts was found in concentration dependent manner. The IC50 values for different radicals were determined and from the IC50 values, it was observed that among the seabuckthorn byproducts, leaves had lowest IC50 value for all the free radicals and was better scavenger of these radicals. The reducing power of the extracts was also in dose dependent manner. The leaves showed better reducing power ability as compared to other extracts.

Keywords: Seabuckthorn, antioxidant, superoxide scavenging, free radical scavenging, Vitamin C, Vitamin E, mineral

Introduction
Bio-based products involved in therapeutic and curative applications in human health is on the rise for the last few decades due to the side effects of synthetic compounds used in medical and nutritional applications (Gupta *et al.*, 2011) [21]. There are numerous reports regarding beneficial effects of fruits, vegetables and other plant derived products on the human health because of the presence of various bioactive molecules in them (Michel *et al*., 2012; Crozier *et al*., 2009) [39, 12]. These substances are secondary metabolites, biosynthesized within plants, mainly phenolic compounds including flavonoids, phenolic acids and tannins having strong antioxidant and antibacterial activity (Saleem *et al*., 2010) [49]. Dietary intake of such phytochemicals may be an important strategy for inhibiting or delaying of pathological conditions caused by free radicals either formed by cellular metabolism, exogenous chemicals or due to stress and is capable of oxidising biomolecules which may cause many diseases (Upadhyay *et al*., 2010) [54]. The biomolecules obtained from the natural plants are in big demands because they have no or less side effects, toxicity of food by synthetics and cosmetic preservatives (Darbre *et al*., 2002) [13]. Sea buckthorn ((*Hippophae rhamnoides* L) is a berry-bearing, hardy bush of the family *Elaeagnaceae*, naturally distributed in Asia and Europe and also introduced in North and South America. It includes 6 species and 12 subspecies, of which Hippophaė rhamnoides, commonly known as sea buckthorn, sandthorn or seaberry is a unique plant, currently being domesticated in several parts of the world (Li, 2003; Li & Schroeder, 1996; Rousi, 1971) [31, 32, 48].

It is a hardy plant, drought and cold resistant, useful for land reclamation and farmstead protection through its vigorous vegetative reproduction and strong, complex root system with nitrogen-fixing nodules (Rongsen, 1992) [46].

Sea buckthorn is an optimal pioneer fascinating plant, the berries has been used for medicinal and nutritional purposes in Russia, Europe, and Asia for many centuries. This future food source, has been gaining attention because of its nutritional benefits as it has been reported to contain more than 190 compounds in the seeds, pulp, fruit and juice. These compounds include fat soluble vitamins (A, K, and E), fatty acids, lipids, organic acids, amino acids, carbohydrates, vitamins C, B1, B2, folic acid, tocopherols and flavanoids, phenols, terpenes and tannins. Many of the substances that found in sea buckthorn are known to have beneficial effects on health (Li & Wang, 1998) [33]. It has been well established in the literature that berries and seeds contain high amounts of natural antioxidants including ascorbic acid, tocopherols, carotenoids, flavonoids, as well as health beneficial fatty acids (Gao *et al.*, 2000; Kallio *et al*., 2002; Rosch, *et al*., 2003) [17, 26, 47]. In spite of several importance of whole sea buckthorn plant, the most important part are berries, from which the juice is extracted and that is the reason why the sea buckthorn berries gain popularity in whole world (Beveridge, *et al*., 1999).

In India the plant inhabits dry temperate region and high-altitude regions of Himachal Pradesh, Jammu and Kashmir and Uttarakhand. Seabuckthorn has recently gained in interest for its nutritional and medicinal values (Diaz 2005; Chawla *et a*l., 2007) [14, 10]. Fruits and leaves are considered to be good source of large number of bioactive substances such as vitamins, trace elements, amino acids, β-carotene, zeaxanthin, lycopene, flavonoids, folic acid, fatty acid, tannic acid etc. These substances are mainly responsible for various pharmacological activities. The phytochemical composition of Seabuckthorn has been found to vary with the origin, climate and method of extraction (Crozier *et al*., 2009) [12]. Its fruit is source of nearly 190 bioactive substances, whereas its oil has nearly 106 such components (Varshneya & Ghabru, 2011) [56]. There is an ample quantity of quality vitamins in Seabuckthorn fruit and leaves. The mineral contents of Seabuckthorn make the shrub most important. Polyphenols present in Seabuckthorn has antioxidant properties and can be used against the damaging effect of free radicals. The phytochemical composition of Seabuckthorn has been found to vary with the origin, climate and method of extraction (CAST, 2002; Bhatnagar *et al*., 2003; Azab *et al*., 2005; Chauhan *et al*., 2013; Ghabru *et al*., 2018) [7, 6, 3, 9, 18].

All this indicates vast potential of sea buckthorn berries as a food resource but little work has been carried out on potential of seabuckthorn leaves. Therefore, in the present literature the chemical and medicinal constituents of sea buckthorn leaves and various processing methods and their effect on nutritive value have been discussed so as to get a clear concept over the compositional importance for the future nutritional research. Study the effects of various dietary levels of seabuckthorn byproducts as bioenhancer.

Materials and Methods

Collection of byproducts of seabuckthorn plant

The different byproducts of Seabuckthorn (*Hippophae rhamnoides* L) *i.e.* leaves, seedcake, pomace with seeds and pomace without seeds were used for *in vitro* study. The leaves and pomace were collected from Keylong region of Himachal Pradesh, India during the month of October. Whereas, seedcakes were collected from the Department of Nutrition, COVAS, CSKHPKV, Palampur, India.

Preparation of extract

The leaves and pomace were air dried in shade. After shade drying, the pomace was divided into two types *i.e.* pomace with seeds and pomace without seeds. The dried leaves and pomace were then powdered in the mixer and stored at the room temperature (18-22 ºC) till further process. The powder of different byproducts was soaked in different solvents [100% Methanol, 70% methanol (aqua-methanol), 50% methanol and 100% aqueous] for 24 hours and kept at room temperature with intermittent shaking. The mixture was then filtered through filtered paper and the extracts were prepared after drying the filterate in rotatary vacuum evaporator at 40 ºC. The percentage of recovery was calculated after determining the weight of the extracts. Finally, the dried extracts were lyophilized and stored at 4 ºC till analysis of *in vitro* antioxidant parameters.

Estimation of total phenol

Total phenolic content in different extracts of Seabuckthorn byproducts was estimated by using Folin-Ciocalteu phenol reagent (FCR) based assay (Gülçin 2012) [20]. Total phenolic content in the extracts of Seabuckthorn byproducts were calculated from the standard curve of gallic acid.

Estimation of total flavonoid

Total flavanoids were estimated spectrophotometrically according to the method of Makkar (2003) [37] with slight modification. Rutin was used as a standard for constructing a calibration curve.

Estimation of β-Carotenoids

β-carotenoids were estimated according to the method of Ranjith *et al*. (2006) [43]. The concentration of β-carotenoid in extracts was obtained using standard curve of beta carotene.

Estimation of Vitamin E

Vitamin E was estimated by the method as described by Kallio *et al*. (2002) [27] with slight modification. For preparation of standard and blank a-tocopherol and distilled water were used and absorbance was measured at 536 nm.

Estimation of Vitamin C

Vitamin C was estimated by 2,4 dinitro phenylhydrazine (DNPH) method as described by Gutzeit *et al*., (2008) [23]. The optical density was recorded at 505 nm against disilled water. Ascorbic acid was used as a standard for calculations.

Estimation of Lycopene

Lycopene contents in seabuckthorn byproducts were measured by the method of Liu *et al*., (1989) [34] and absorbance read in a spectrophotometer at 503 nm.

Identification and quantification of marker compounds using HPLC

Flavonoid profiling of various Seabuckthorn by products was got done from IHBT, CSIR research center, Palampur, H.P using HPLC system consisted of a Shimadzu HPLC (Model LC-20AT pump, DGU-20A5 degasser) equipped with photo-

diode array detector (CBM-20A; Shimadzu, Kypto, Japan) interfaced with an IBM Pentium 4 personal computer. The separation was performed on a Phenomenex Luna C-18 column (250 × 4.6 mm i.d., 5µm). The temperature of the column was set at 25˚C. Elution of samples (20 µl) was performed with gradient solvent programme, at a flow rate of 1 ml/min for 30 minutes. The mobile phase consisted of 0.05% triflouroacetic acid in water (A) and acetonitrile (B) with following gradient: 15-60% B in 0-30 min and 15% B in 35 min. The detection was done at 355 nm. The identification of compounds was performed on the basis of retention time, coinjection and spectral matching with standards.

Proximate analysis

Proximate analysis of Seabuckthorn byproduct was done as per method given in AOAC (1995) [2].

Mineral content

Mineral content was estimated by the method of Indrayan *et al*. (2007) [25] using atomic absorption spectrophotometer, Analyst 400, Perkin Elmer.

Measurement of Total Antioxidant Activity

The total antioxidant activity of different extracts was determined according to the method of Re *et al*. (1999) [44] based on ABTS•+ scavenging assay. The radical scavenging capacity was performed by mixing 30 µl of the extract (sample) into 3.0 ml of ABTS•+ solution. After proper mixing, the absorbance was recorded at 734 nm after 3 minutes against distilled water. A control solution of 30 µl 70% methanol in 3.0 ml of ABTS•+ solution was also prepared and analyzed. The percentage of inhibition of ABTS•+ radicals at different concentrations were determined by using the following formulae:

% ABTS•+ inhibition = [1–(A734nm Sample/A734nm Control)] x 100

Free radical scavenging activity

The potential of extracts to scavenge DPPH radicals was determined according to the method of Gordon *et al.* (1990) [19]. The absorbance of the samples and control solutions were determined at 517 nm against water and the % DPPH radical scavenging activity was calculated as follows:

% DPPH radical scavenging activity = [1– (A517nm sample/A517nm control)] x 100

Superoxide Anion Radical Scavenging Assay

The superoxide anion radical-scavenging ability of extract was assessed by the method described by Gordon *et al* (1990) [19] followed by slight modification.

Percentage inhibition of the superoxide anion radicals was calculated using the following equation:

% superoxide radical scavenging activity = [1–(A560nm sample/A560nm control)] x 100

Hydroxyl radical scavenging assay

The potential of different concentrations of Seabuckthorn byproducts to scavenge the hydroxyl radical generated by the Fenton reaction was measured according to the method of Aruoma & Cuppet (1997) [1]. Absorbance was measured at 532 nm. From the absorbance the % scavenging activity was calculated using the following formula.

% Hydroxyl radical scavenging activity = [1– (A532nm sample/A532nm control)] x 100

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured by the method of Aruoma & Cuppet (1997) [1] with slight modification by using Griess' reagent. The absorbance was measured at 546 nm. From the absorbance the % scavenging activity was calculated using the following formula.

% Nitric oxide radical scavenging activity = [1-(A546nm sample/A546nm control)] x 100

Reducing power assay

Reducing power of different extracts of seabuckthorn byproducts were determined by the method of Rice-Evans *et al*., (1996) [45] with slight modification. Absorbance was measured at 700 nm.

Statistical analysis

The data were analyzed using Graph Pad Instat for windows (Graph Pad Software, San Diego, California, USA) and the significant difference between means was determined using Tukey-Kramer multiple comparison test.

Results

In vitro study on seabuckthorn (*Hippophae rhamnoides*) byproducts was carried out to evaluate their antioxidant capacity and chemoprofiling.

The percentage of recovery was calculated after determining the weight of the extracts (Table 1) and the maximum recovery (16%) was found in 70% methanolic extract of seabuckthorn leaves.

Table 1: Percentage of recovery of different extracts of Seabuckthorn byproducts

Sample	Type of Extract		
	100% Methanolic	70% Methanolic	100% Aqueous
Leaves	14	16	12
Seedcake	10	12	8
Pomace with seeds	11	10	8
Pomace without seeds	10	10	4

The data on total phenols, total flavonoids, Vitamin C and Vitamin E content of all the byproducts of seabuckthorn is presented in Table 2. Among all byproducts the leaves extract contained significantly high amount of total phenols (332.49±7.45mg/g), total flavonoids (271.56±5.41mg/g), β-crotenoids (262.200±17.48 µg/mg), vitamin C (399.84±4.90mg/100g) and lycopene content (8.50±2.92 mg/100g) whereas Pomace with seeds contained high amount of vitamin E (234.00±0.02mg/100g).

Table 2: Total phenols, total flavonoids, β-crotenoids, vitamin C, vitamin E and lycopene content of Seabuckthorn byproducts extracted in 50% methanol

Sample	Total phenols mg/g	Total Flavonoid mg/g	p-Carotenoid µg/mg	Vitamin C mg/100g	Vitamin E mg/100g	Lycopene mg/100g
Leaves	332.49±7.45[a]	271.56±5.41[a]	262.200±17.48[a]	399.84±4.90[a]	216.00±0.02[c]	8.50±2.92[a]
Seedcake	183.75±14.98[c]	126.07±2.51[c]	52.440±15.138[c]	206.27±5.52[d]	76.00±0.02[d]	0.30±0.01[d]
Pomace with seeds	227.04+4.17[b]	202.12±9.31[b]	262.200±15.13[a]	224.40±2.08[c]	234.00±0.02[a]	2.06±0.005[c]
Pomace without seeds	184.28±1.58[c]	128.55±2.19[c]	139.840±23.12[b]	249.33±5.23[b]	224.00±0.03[b]	6.64±0.012[b]

Values are expressed as Mean ±SEM. The means with same superscripts in between columns do not differ significantly at 5% level.

The proximate analysis of nutritive contents of Seabuckthorn byproducts are depicted in Table 3. The ash content which is an index of mineral contents, ranged from 1.3 to 4%. The moisture content was significantly highest in seedcake. The crude protein contents ranged from 13.89 to 23% and recorded highest in leaves (22.09%) and seedcake (23%). Ether and crude fibre contents were recorded highest in pomace with seeds.

Table 3: Nutritional parameters of Seabuckthorn byproducts

Sample	Ash (%)	Moisture content (%)	Dry matter (%)	Protein (%)	Ether (%)	Crude Fibre (%)
Leaves	4[a]	9[b]	89[b]	22.09[a]	9[b]	1[d]
Seedcake	4[a]	10[a]	90[b]	23[a]	9[b]	13[c]
Pomace with seeds	1.3[c]	6.81[c]	93.19[a]	14.01[b]	20.50[a]	30.60[a]
Pomace without seeds	2[b]	7[c]	93[a]	13.89[b]	19.87[a]	28.89[b]

Values are expressed as Mean ±SEM. The means with same superscripts in between columns do not differ significantly at 5% level.

The mineral composition in Seabuckthorn byproducts are shown in Table 4. The concentrations of sodium (Na) ranging from 40 to 160 mg/g. Among all the byproducts leaves contained high concentration of all the minerals estimated except Zn.

Table 4: Mineral composition of Seabuckthorn byproducts

Sample	Na g/g	K mg/g	Ca mg/g	Mg mg/g	Zn mg/g	Fe mg/g
Leaves	160	7.64	10.32	1.716	0.02	0.720
Seedcake	80	4.89	0.155	1.4	0.083	0.398
Pomace with seeds	44	4.43	0.362	0.551	0.029	0.290
Pomace without seeds	40	3.49	0.298	0.421	0.020	0.189

In vitro antioxidant activity of seabuckthorn byproducts were evaluated through ABTS (2,2 azonobis 3 ethylene benzothiozoline 6 sulphonic acid) radical, nitric oxide radical, DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical, hydroxyl radical, superoxide radical anion scavenging activities and reducing power assay. The free radical scavenging activity of all the extracts and different concentrations of byproducts increased in a concentration dependent manner. The IC50 values of leaves extract from the ABTS radical, nitric oxide radical, DPPH radical, hydroxyl radical, superoxide radical anion scavenging assay were lowest as compared to others as given in tables 5, 6, 7, 8, 9 and 10. The leaf extract showed lowest IC50 value in all the parameters as compared to other byproducts and hence possessed high antioxidative activity.

Table 5: The IC50 value (mg/ml) of different extracts of Seabuckthorn byproducts for Total Antioxidant Activity and the values are expressed as Mean ±SE. (n=3)

Sample	Type of Extract		
	100% Methanolic	70% Methanolic	100% Aqueous
Leaves	0.346±0.023	0.365±0.019	0.555±0.025
Seedcake	0.369±0.028	0.482±0.033	1.005±0.042
Pomace with seeds	0.717±0.049	0.802±0.032	2.050±0.099
Pomace without seeds	2.072±0.253	4.138±0.424	3.210±0.246

Table 6: The IC50 value (µg/ml) of different extracts of Seabuckthorn byproducts for Free Radical Scavenging Activity and the values are expressed as Mean ±SE. (n=3)

Sample	Type of Extract		
	100% Methanolic	70% Methanolic	100% Aqueous
Leaves	44.92±2.77	42.11±2.15	52.32±1.69
Seedcake	42.25±3.35	45.26±3.47	159.91±4.91
Pomace with seeds	105.62±21.35	120.61±21.89	199.82±17.44
Pomace without seeds	179.77±33.67	143.33±14.24	586.24±64.05

Table 7: The IC50 value (µg/ml) of different extracts of Seabuckthorn byproducts for Superoxide Radical Scavenging Activity and the values are expressed as Mean ±SE. (n=3)

Sample	Type of Extract		
	100% Methanolic	70% Methanolic	100% Aqueous
Leaves	146.49±19.47	119.15±8.37	191.31±12.75
Seedcake	177.90±22.25	155.09±3.26	278.51±25.77
Pomace with seeds	149.85±16.25	142.15±14.43	207.88±13.53
Pomace without seeds	287.97±60.36	240.45±46.73	321.36±38.87

Table 8: The IC50 value (µg/ml) of different extracts of Seabuckthorn byproducts for Hydroxyl (OH) Radical Scavenging Activity and the values are expressed as Mean ±SE. (n=3)

Sample	Type of Extract		
	100% Methanolic	70% Methanolic	100% Aqueous
Leaves	14.76±1.81	18.48±1.77	58.00±6.76
Seedcake	20.24±0.46	22.19±1.26	127.50±23.09
Pomace with seeds	16.67±0.31	19.34±0.45	229.25±22.85
Pomace without seeds	29.77±0.94	30.49±1.42	183.81±15.50

Table 9: The IC50 value (µg/ml) of different extracts of Seabuckthorn byproducts for Nitric oxide (NO) Radical Scavenging Activity and the values are expressed as Mean ±SE. (n=3)

Sample	Type of Extract		
	100% Methanolic	70% Methanolic	100% Aqueous
Leaves	44.25±4.30	45.26±5.43	54.22±2.34
Seedcake	49.97±1.18	52.06±2.50	174.85±7.37
Pomace with seeds	82.61±5.06	61.99±4.25	192.58±19.40
Pomace without seeds	235.69±25.23	226.84±15.51	358.05±32.19

Table 10: The reducing power capability of different extracts of Seabuckthorn byproducts and the values are expressed in absorbance as Mean ±SE. (n=3)

Conc. of extract	Leaves	Seedcake	Pomace with seeds	Pomace without seeds
10	0.27±0.01	0.28±0.01	0.21±0.01	0.21±0.01
20	0.37±0.01	0.32±0.01	0.23±0.01	0.23±0.01
40	0.48±0.00	0.44±0.01	0.26±0.02	0.26±0.02
60	0.59±0.01	0.55±0.00	0.29±0.02	0.29±0.02
80	0.72±0.00	0.59±0.01	0.32±0.02	0.32±0.02
100	0.84±0.01	0.65±0.01	0.37±0.03	0.37±0.03
200	0.86±0.01	0.76±0.02	0.46±0.04	0.46±0.04

Chemical constituent's i.e. total phenol, total flavonoid, β-carotene and lycopene contents were found to be higher in methanolic extracts of leaves as compared to other byproducts. The HPLC analysis of the various seabuckthorn byproducts for the different flavonoids was shown that rutin was present in the extracts of leaves and pomace. Quercetin-3-galactoside was present in the extracts of leaves and 100% and 70% methanolic extracts of pomace without seeds, whereas, isorhamnetin was present in the 100% and 70% methanolic extracts of pomace.

Table 11: Concentration of different flavonoids in different extracts of seabuckthorn byproducts. (Yield %±CV %)

Type of extract	Different flavonoids (Yield %±CV %)					
	Rutin	Quercetin-3-galactoside	Myricetin	Quercetin	Kaempferol	Isorhamnetin
Leaves (100% methanolic)	0.2542±2.13	0.2739±1.63	-	-	-	-
Leaves (70% methanolic)	0.4678±2.08	0.5712±2.80	-	-	-	-
Leaves (100% Aqueous)	0.3585±2.28	0.4436±1.01	-	-	-	-
Seedcake (100% methanolic)	-	-	-	-	-	-
Seedcake (70% methanolic)	-	-	-	-	-	-
Seedcake (100% Aqueous)	-	-	-	-	-	-
Pomace with Seed (100% methanolic)	0.0532±0.68	-	-	-	-	0.0149±3.92
Pomace with seed (70% methanolic)	0.1188±3.76	-	-	-	-	0.0172±4.65
Pomace with seed (100% Aqueous)	0.0438±2.44	-	-	-	-	-
Pomace without seed (100% methanolic)	0.0507±1.53	0.0278±3.30	-	-	-	0.0140±2.89
Pomace without seed (70% methanolic)	0.0644±3.89	0.0346±3.18	-	-	-	0.0123±3.37
Pomace without seed (100% Aqueous)	0.0406±3.87	-	-	-	-	-

A perusal of Table 12 indicates the total phenols, total flavonoids, Vitamin C and Vitamin E content of seabuckthorn leaves powder extracted in different solvents. 50% Actone extract contained maximum amount of all the chemical constituents followed by 70% acetone extraction.

Table 12: Total phenols, Total flavonoids, Vitamin C and Vitamin E content of seabuckthorn leaves powder extracted in different solvents

S. No.	Extracts	Total phenols (mg Gallic acid/gm extract)	Total flavonoids (mg Rutin/gm extract)	Vitamin C (g/100 gm extract)	Vitamin E (g/100 gm extract)
1.	70% Acetone	494.281±74.343	116.208±3.005	12.721±1.532	69.81±3.937
2.	50% Acetone	767.964±26.325	142.458±7.982	14.002±0.970	69.927±5.178
3.	70% Methanol	399.844±66.147	106.208±8.333	5.768±1.049	66.11±10.213
4.	50 % Methanol	442±48.664	112.458±6.821	11.174±0.666	64.31±3.955

Values are expressed as Mean ± SEM (n=3)

Table 13: IC50 values of different extracts of Seabuckthorn leaves for Total antioxidant activity, free radical scavenging activity

Sr. No.	Antioxidant activity	IC50 value (µg/ml)			
		70% Acetone	50% Acetone	70% Methanol	50% Methanol
1.	Total antioxidant activity	27.110±0.57	16.551±0.233	26.353±0.566	49.564±0.643
2.	Free radical scavenging activity	0.983±0.297	0.669±0.205	1.298±0.030	1.586±0.263

Values are expressed as Mean ± SEM (n=3)

Finally, it could be concluded that different byproducts of seabuckthorn have antioxidant properties and on the basis of economics, antioxidant potentials and chemofrofiling, leaves will be selected for developing poultry nutraceuticals. The chemoprofiling of the various seabuckthorn byproducts for total flavonoids, total carotenoids, vitamin C, Vitamin E has indicated that leaves and pomace with seeds of seabuckthorn possess good amount of carotenoids, flavanoids, and vitamins.

Discussion

Chemoprofiling (Total phenols, total flavonoids, vitamin C, vitamin E, lycopene and β-carotene contents) of various seabuckthorn byproducts has been done and it was found that among all the seabuckthorn byproducts, leaf extract contained significantly highest amount of total phenols (332.49±7.45 mg/g), total flavonoids (271.56±5.41 mg/g), vitamin C (399.49±4.90 mg/100g) and lycopene content (8.50±2.92 mg/100g). Bioactive substances like vitamins (A, C, E, riboflavin, folic acid and K), carotenoids (α, β-carotene, and lycopene), flavonoids, organic acids (malic acid and oxalic acid), sterols (ergosterol, stigmasterol, lanosterol, and amyrins) and some essential amino acids present in all parts of the plant is reported by several workers earlier (Hakkinen *et al*., 1999; Upendra *et al*., 2008) [24, 55]. Zheng and Song (1992) [59] found that SBT fresh leaves are rich in total carotenoids (26.3 mg/100g) and total chlorophyll (98.8 mg/100g), an indicator of quality for green vegetables; whereas dried leaves still contained large quantities of bioactive compounds comparable to commonly consumed vegetables. *Hippophae* leaves also contain significant amounts of proteins (20.7%), amino acids (0.73% lysine, 0.13% methionine and cystine) [Varshneya and Ghabru 2011] [56], minerals (Ca, Mg and K), folic acid, catechins, esterified sterols, triterpenols and isoprenols (Zeb 2004; Wani *et al*., 2013] [58, 57]. According to Kumar *et al*. (2011) [29], the tannins hippo-phaenins A and B were isolated from SBT leaves.

The leaves of sea buckthorn are rich in kaempferol-3-O- β-D-(6"-O-coumaryl) glycoside, 1-feruloyl-β-D-glucopyranoside, isorhamnetin-3-O-glucoside, quercetin-3-O-β-D glucopyranoside, quercetin-3-O-β-D-glucopyranosyl-7-O-a-L-rhamnopyranoside, and isorhamnetin-3-O-rutinoside (Table 18). Nine fractions, four monomeric flavan-3-ols, catechin, epicatechin, gallocatechin and epigallocatechin, along with two dimeric procyanidins, catechin(4a-8) catechin and catechin (4a-8) epicatechin, have been reported from the extracts of sea buckthorn seeds (Fan *et al*., 2007; Kim *et al*., 2011; Nitin *et al*., 2010) [16, 30, 41].

The proximate analysis of nutritive contents of Seabuckthorn byproduct was determined. The ash content which is an index of mineral contents, ranged from 1.3 to 4%. The moisture content was significantly highest in seedcake. The crude protein contents ranged from 13.89 to 23% and recorded highest in leaves (22.09%) and seedcake (23%). Ether and crude fibre contents were recorded highest in pomace with seeds. The mineral composition in Seabuckthorn byproducts are shown in Table 5. High concentrations of sodium (Na) were present, ranging from 40 to 160 mg/g. Among all the byproducts leaves contained high concentration of all the minerals estimated.

The *in vitro* antioxidant assays result indicated that the all byproducts of seabuckthorn have strong potential to act as antioxidant (Tables 11-17). The assays like 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP), which are often used to test the antioxidant activity, have revealed that the antioxidant activity of seed and root extracts is better than that of leaf and stem extracts (Surya Kumar & Gupta 2011; Nitin *et al*., 2010) [21, 41]. Gallic acid, which is also present in sea buckthorn, has been reported to be the most effective antioxidant (Pandurangan, *et al*., 2011) [42]. The antioxidant potential of aqueous extract of sea buckthorn leaves varies within the range of 76.44-88.82% while as the total polyphenols vary in the range of 67.91-88.69 GAE/g (Wani *et al*., 2013) [57]. Sea buckthorn leaf evaluation using maceration, Soxhlet and subcritical water extraction techniques showed the antioxidant potential of 86.35, 133.31-255.87, and 164.03-343.86 Trolox equivalents per gram (TE/g), respectively, while as the respective total phenolic content was reported to be 28.35, 43.77-77.85 and 60.22-86.70 mg/g (Kumar *et al*., 2011) [29]. The phenolic rich fraction (PRF) of sea buckthorn leaves showed a total phenolic content of 319.33 mg gallic acid equivalents (GAE) per gram while as in the berries, it ranged from 21.31 to 55.38 mg GAE/g on dry weight basis. It showed the highest antioxidant activity of 93.54% and the lowest of 80.38% with no correlation between the total phenolic content and the antioxidant activity. The DPPH radical scavenging activity of sea buckthorn leaf extract (50% effective concentration (EC50) = 1.81 µg/mL) is higher than the butanol fraction (EC50 = 1.86 µg/mL) and quercetin-3-O-β-D-glucopyranoside. It showed stronger reducing power (OD700 = 1.83, and 1.78, respectively), with the highest amount of phenolic compounds (477 mg GAE/g) contained in the butanol fraction (Ercisli *et al*., 2007; Kim *et al*., 2011;

Maheshwari *et al*., 2011) [15, 30, 36]. The EC50 values of sea buckthorn seed oil from the hydrogen peroxide, superoxide radical, and hydroxyl radical scavenging assays were 2.63, 2.16 and 0.77 mg/ml, respectively (Ting *et al*., 2011) [53]. Taken together, sea buckthorn seed oil, leaf, branches, and root extracts have significant potential as natural antioxidants and could be used potentially for food additives and the development of useful natural compounds.

Phenols are the major plant compounds with antioxidant activity, which is believed to be mainly due to their redox properties, that plays an important role in adsorbing and neutralizing the free radicals, quenching singlet and triplet oxygen, or decomposition of the peroxides (Long *et al*., 2000) [35]. In the present study, total phenolics and flavonoids contents were determined to analyze the chemical composition of *Hippophae rhamnoides* leaves. Results showed that phenolic and flavonoids compounds were present in considerable amount in the *Hippophae rhamnoides* leaf extract. This shows that the *Hippophae rhamnoides* leaf extracts possess antioxidant properties that can help in restoring the health of humans by causing inhibition of oxidative damage diseases. The information about the total phenolic levels in *Hippophae rhamnoides* leaves supplement the view point of various workers who demonstrated polyphenols as one of the important contributors to the antioxidant and free-radical scavenging activities of various plant extracts. Our findings are in conformity with other studies on different medicinal plants and herbs (Kevers *et al*., 2007; Sreeramulu and Raghunath, 2010) [28, 51].

The free radical scavenging activity of *Hippophae rhamnoides* leaf extracts was studied by their ability to decolourize the stable ABTS and DPPH free radicals, which provides information on the reactivity of compounds with a stable free radical (Badami *et al*., 2003) [4]. The results of this study showed that *Hippophae rhamnoides* leaf extracts are effective in scavenging ABTS and DPPH radicals, though the ABTS and DPPH radical scavenging abilities of the extracts were significantly less than those of ascorbic acid. This indicates that the extracts have the proton-donating or scavenging ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Results obtained from this assay further supported the validity of DPPH and ABTS assay and reconfirms the antioxidant potential of the *Hippophae rhamnoides* leaf extracts. Significant antioxidant activityshowed by *Hippophae rhamnoides* leaf extract provide a scientific validation for the traditional use of these plants in traditional medicine system, however, work on isolation and identification of active compounds and its efficacy needs further investigations.

However, it is observed that *Hippophae rhamnoides* fruit juice with a few phenolic compounds also exhibited good antioxidant capacity but their contribution to the antioxidant effect is very low as compared to ascorbic acid (Rosch *et al*., 2003) [47]. The antibacterial activity of chloroform, ethyl acetate, acetone and methanol extracts of *Hippophae rhamnoides*seeds was also studied (Rosch *et al*., 2003; Negi *et al*., 2005) [47, 40]. Similarly, Chauhan *et al*. (2007) [8] showed antioxidant and antibacterial activities of aqueous extract of seabuckthorn seeds. Seed oil of *Hippophae rhamnoides* possesses several strong antioxidative and antimicrobial properties, which are due to high content of tocopherols and carotenoids present in the oil (Chen *et al*., 1990) [11]. In addition, antiviral and other biological activities of *Hippophae rhamnoides* leaf extracts have also been documented by Shipulina (2001) [50]. Research in the development of formula food, pre-food and food additives of SBT should provide for conditions of great potential and markets.

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References

1. Aruoma, Cuppet SL. (Eds.). Antioxidant methodology *in vivo* and in vitro concepts. Champaign, IL: AOCS Press; c1997. p. 2-29.
2. AOAC. Official methods of analysis 16th Ed. Association of official analytical chemists. Washington DC, USA; c1995.
3. Azab RM, Tawakkol WM, Hamad AM, Abou-Elmagd MK, El-Agrab HM, Refai MK. Detection and estimation of aflatoxin B1 in feeds and its biodegradation; c2005.
4. Badami S, Gupta MK, Suresh B. Antioxidant activity of the ethanolic extract of *Striga orobanchioides*. J Ethnopharmacol. 2003;85:227-230.
5. Beveridge T, Harrison JE, Drover J. Processing effects on the composition of sea buckthorn juice from *Hippophaė rhamnoides* L. cv. Indian Summer. Journal of Agricultural and Food Chemistry. 2002;50:113-115.
6. Bhatnagar D, Ehrlich KC, Cleveland TE. Molecular genetic analysis and regulation of aflatoxin biosynthesis, Appl. Microbiol. Biotechnol. 2003;61:83-93.
7. CAST (Council for Agricultural Science and Technology). Mycotoxins: Risks in Plant Animal and Human Systems. Ames, Iowa: CAST, 2002, 199.
8. Chauhan AS, Negi PS, Ramteke RS. Antioxidant and antibacterial activities of aqueous extract of Seabuckthorn (*Hippophae rhamnoides*) seeds. *Fitoterapia*. 2007;78:590-592.
9. Chauhan S, Varshneya C, Ghabru A. *In vitro* and *in vivo* antioxidant activities of seabuckthorn berries and seed oil. Journal of Cell and Tissue Research. 2013;13(1):3525-3520.
10. Chawla R, Arora R, Singh S, Sagar RK, Sharma RK, *et al*., Radioprotective and antioxidant activity of fractionated extracts of berries of *Hippophae rhamnoides*. J Med. Food. 2007;10:101-109.
11. Chen YD, Jiang ZR, Qin WI, Ni MN, Li XL. Research on chemical composition and characteristics of Sea buckthorn berry and its oil. Chem. Indus. Fores. Prod. 1990;10:163-175.
12. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: Chemistry, bioavailability and effects on health. Nat. Prod. Rep. 2009;26:1001-1043.
13. Darbre PD, Byford JR, Shaw LE, Horton RA, Pope GS, Sauer MJ. Oestrogenic activity of isobutylparaben *In vitro* and *In vivo*. J Appl. Toxicol., 2002;22:219-226.
14. Diaz DE. The Mycotoxin Blue Book. Nottingham University Press. Nottingham, England; c2005.
15. Ercisli S, Orhan E, Ozdemir O, Sengul M. The genotypic effects on the chemical composition and antioxidant activity of sea buckthorn (*Hippophae rhamnoides* L.) berries grown in Turkey. Scientia Horticulturae. 2007;115:27-33.

16. Fan J, Ding X, Gu W. Radical-scavenging proanthocyanidins from sea buckthorn seed. Food Chemistry. 2007;102(1):168-177.
17. Gao X, Ohlander M, Jeppsson N, Bjork L, Trajkovski V. Changes of antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophaë rhamnoides* L.) during maturation. Journal of Agricultural and Food Chemistry. 2000;48(5):1485-1490.
18. Ghabru A, Chauhan S, Varshneya C. Effect of Seabuckthorn leaves on Antioxidant and Microsomal Enzymes in poultry birds. The Journal of Phytopharmacology. 2018;7(5):440-445.
19. Gordon MH. The mechanism of antioxidant action *in vitro*. In Hudson BJF (Ed.), Food antioxidants. London: Elsevier Science; c1990. p. 1-18.
20. Gülçin İ. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2012;86:345-391. https://doi.org/10.1007/s00204-011-0774-2
21. Gupta SM, Gupta AK, Ahmed Z, Kumar A. Antibacterial and antifungal activity in leaf, seed extract and seed oil of Seabuckthorn (*Hippophae salicifolia* D. Don) plant. J Plant Pathol. Microbiol. 2011;2:105-108.
22. Gupta R, Flora SJ. Therapeutic value of *Hippophae rhamnoides* L. against subchronic arsenic toxicity in mice. Journal of Medicinal Food. 2005;8:353-361.
23. Gutzeit D, Baleanu G, Winterhalter P, Jerz G. Vitamin C content in sea buckthorn berries (*Hippophaë rhamnoides* L. ssp. rhamnoides) and related products: A kinetic study on storage stability and the determination of processing effects. Journal of Food Science. 2008;73(9):615-620.
24. Hakkinen SH, Karenlampi SO, Heinonen IM, Mykkanen HM, Torronen AR. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. Journal of Agricultural and Food Chemistry. 1999;47:2274-2279.
25. Indrayan AK, Sharma S, Durgapal D, Kumar N, Kumar M. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Current Science. 2005;89:1252-1255.
26. Kallio H, Yang B, Peippo P. Effects of different origins and harvesting time on vitamin C, tocopherols and tocotrienols in sea buckthorn (*Hippophaë rhamnoides*) berries. Journal of Agricultural and Food Chemistry. 2002;50(21):6136-6142.
27. Kallio H, Yang B, Peippo P, Tahvonen R, Pan R. Triacylglycerols, glycerophospholipids, tocopherols, tocotrienols in berries and seeds of two sspecies (ssp. sinensis and mongolica) of sea buckthorn (*Hippophaë rhaminoides*). Journal of Agricultural and Food Chemistry. 2002;50(10):3004-3009.
28. Kevers C, Falkowski M, Tabart J, Defraigne JO, Dommes J, Pincemail J. Evolution of antioxidant capacity during storage of selected fruits and vegetables. J Agric. Food Chem. 2007;55:8596-8605.
29. Kumar MSY, Dutta R, Prasad D, Misra K. Subcritical water extraction of antioxidant compounds from Seabuckthorn (*Hippophae rhamnoides*) leaves for the comparative evaluation of antioxidant activity. Food Chemistry. 2011;127:1309-1316.
30. Kim HI, Kim MS, Lee KM, Park SK, Seo KI, Kim HJ, *et al*. Anti-visceral obesity and antioxidant effects of powdered sea buckthorn (*Hippophae rhamnoides* L.) leaf tea in diet-induced obese mice. Food and Chemical Toxicology. 2011;49:2370-2376.
31. Li TSC. Taxonomy, natural distribution and botany. In Li TSC & Beveridge T (Eds.), Sea buckthorn (*Hippophaë rhamnoides* L.): Production and utilization. Ottawa, ON: NRC Research Press, 2003, 7-11.
32. Li TSC, Schroeder WR. Sea buckthorn (*Hippophaë rhamnoides* L.): A multipurpose plant. Hort. Technology. 1996;6:370-380.
33. Li TSC, Wang LCH. Physiological components and health effects of ginseng, echinacea and sea buckthorn. In G. Mazza (Ed.), Functional foods, biochemical and processing aspects. Lancaster, PA: Technomic Publishing Company Inc.; c1998. p. 329-356.
34. Liu D, Zhang H, Zhang S, Kou X, Liu R, Wang S. A food additives "sea buckthorn yellow". Proceedings of international symposium on sea buckthorn (*H. rhamnoides* L.), Xian, China; c1989 Oct, 318-319.
35. Long LH, Kwee DC, Halliwell B. The antioxidant activities of seasonings used in Asian cooking. Powerful antioxidant activity of dark soy sauce revealed using the ABTS assay. Free Rad. Res. 2000;32:181-186.
36. Maheshwari DT, Yogendra Kumar MS, Verma SK, Singh VK, Singh SN. Antioxidant and hepatoprotective activities of phenolic rich fraction of Seabuckthorn (*Hippophae rhamnoides* L.) leaves. Food and Chemical Toxicology. 2011;49;2422-2428.
37. Makkar HPS. Quantification of Tannins in Tree and Shrub Foliage: A Laboratory Manual. Kluwer Academic Publishers, Dordrecht, Netherlands; c2003. p. 43-54. ISBN-13: 9781402016325.
38. Marklund S, Marklund G. Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-474.
39. Michel T, Destandau E, Le Floch G, Lucchesi ME, Elfakir C. Antimicrobial, antioxidant and phytochemical investigations of sea buckthorn (*Hippophaë rhamnoides* L.) leaf, stem, root and seed. Food Chemistry. 2012;131:754-760.
40. Negi PS, Chauhan AS, Sadia GA, Rohinishree YS, Ramteke RS. Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) seed extracts. Food Chem. 2005;92:119-124.
41. Nitin K, Upadhyay MS, Kumar Y, Gupta A. Antioxidant, cytoprotective and antibacterial effects of sea buckthorn (*Hippophae rhamnoides* L.) leaves. Food and Chemical Toxicology. 2010;48:3443-3448.
42. Pandurangan N, Bose C, Banerji A. Synthesis and antioxygenic activities of sea buckthorn flavone-3-ols and analogs. Bioorganic & Medicinal Chemistry Letters. 2011;21:5328-5330.
43. Ranjith A, Kumar KS, Venugopalan VV, Arumughan C, Sawhney RC, Singh V. Fatty acids, tocols and caratenoides in pulp oil of three sea buchthorn species (*H. rhamnoides*, *H. salicifolia* and *H. tibetana*) grown in the Indian Himalayas. J Am. Oil Chem. Soc. 2006;83:359-364.
44. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 1999;26:1231-1237.
45. Rice-Evans CA, Miller NJ, Pagana G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology & Medicine.

1996;20:933-956.
46. Rongsen A. Sea buckthorn a multipurpose plant for fragile mountains. ICIMOD occasional paper No. 20, Kathmandu, Nepal; c1992. p. 6-7, 18-20.
47. Rosch D, Bergmann M, Knorr D, Kroh LW. Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. Journal of Agricultural and Food Chemistry. 2003;51:4233-4239.
48. Rousi A. The genus Hippophaë L: A taxonomic study. Annales Botanica Fennici. 1971;8(3);177-277.
49. Saleem M, Nazir M, Ali MS, Hussain YS, Lee N Riaz, Jabbar A. Antimicrobial natural products: An update on future antibiotic drug candidates. Nat. Prod. Rep. 2010;27:238-254.
50. Shipulina LD. A study of antiviral activity and other biological properties of hiporamin. A new antiviral drug from Seabuckthorn leaves. Proceedings of the International Workshop on Sea Buckthorn, Palampur and Indian Society of Tree Scientists, Solan, India; c2001 Feb. p. 212-213.
51. Sreeramulu D, Raghunath M. Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. Food Res. International. 2010;43:1017-1020.
52. Suryakumar G, Gupta A. Medicinal and therapeutic potential of Sea buckthorn (*Hippophae rhamnoides* L.). J Ethnopharmacol. 2011;138:268-278.
53. Ting HC, Hsu YW, Tsai CF, Lu FJ, Chou MC, Chen WK. The *in vitro* and *in vivo* antioxidant properties of seabuckthorn (*Hippophae rhamnoides* L.) seed oil. Food Chemistry. 2011;125:652-659.
54. Upadhyay NK, Kumar MSY, Gupta A. Antioxidant, cytoprotective and antibacterial effects of Sea buckthorn (*Hippophae rhamnoides* L.) leaves. Food Chem. Tox. 2010;48:3443-3448.
55. Upendra KS, Sharma K, Sharma N, Sharma A, Singh HP, Sinha AK. Microwave assisted efficient extraction of different parts of *Hippophae rhamnoides* for the comparative evaluation of antioxidant activity and quantification of its phenolic constituents by reverse phase high performance liquid chromatography (RP-HPLC). Journal of Agricultural and Food Chemistry. 2008;56:374-379.
56. Varshneya C, Ghabru A. Chemoprofiling of Seabuckthorn (*Hippophae rhamnoides*) byproducts. Proceedings of National Conference on Seabuckthorn: Emerging Trends in R&D on Health Protection & Environmental Conservation; c2011 Dec. p. 93-98.
57. Wani TA, Wani SM, Shah AG, Masoodi FA. Optimizing conditions for antioxidant extraction from Sea Buckthorn leaf (*Hippophae rhamnoides* L.) as herbal tea using response surface methodology (RSM). International Food Research Journal. 2013;20:1677-1681.
58. Zeb A. Chemical and nutritional constituents of sea buckthorn juice. Pakistan Journal of Nutrition. 2004a;3(2):99-106.
59. Zheng XW, Song XJ. Analysis of the fruit nutrient composition of nine types of sea buckthorn in Liaoning, China North. Fruits of China. 1992;3:22-24.